

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding carotenoid biosynthetic pathway enzymes, comprising a polynucleotide wherein the genetic organization of the polynucleotide is: *crtE-idi-crtY-crtI-crtB-crtZ*, wherein the *crtE*, *idi*, *crtY*, *crtI*, *crtB*, and *crtZ* genes are clustered in the order stated and wherein the transcription of the *crtZ* occurs in opposite orientation to that of *crtE*, *idi*, *crtY*, *crtI*, and *crtB*.
2. The isolated nucleic acid molecule of Claim 1, wherein at least one of the carotenoid biosynthetic pathway enzymes are encoded by the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, and 12.
3. The isolated acid molecule of Claim 1 having the sequence as set forth in SEQ ID NO:18.
4. An isolated nucleic acid molecule encoding a carotenoid biosynthetic pathway enzyme, selected from the group consisting of:
  - (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, and 12;
  - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
  - (c) an isolated nucleic acid molecule that is complementary to (a) or (b).
5. The isolated nucleic acid molecule of Claim 4 selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, and 11.
6. An isolated nucleic acid molecule as set forth in SEQ ID NO:18, comprising the *crtE-idi-crtY-crtI-crtB-crtZ*, genes or an isolated nucleic acid molecule having at least 95% identity to SEQ ID NO:18, wherein the isolated nucleic acid molecule encodes all of the polypeptides *crtE*, *idi*, *crtY*, *crtI*, *crtB*, and *crtZ*.
7. An isolated nucleic acid fragment of Claim 1 or 4 isolated from *Pantoea agglomerans* strain DC404.
8. A polypeptide encoded by the isolated nucleic acid molecule of Claim 4.
9. The polypeptide of Claim 8 selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, and 12.

10. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a geranylgeranyl pyrophosphate synthetase enzyme of at least 301 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide  
5 having the sequence as set forth in SEQ ID NO:2;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

11. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding an isopentenyl pyrophosphate isomerase enzyme of at least 349 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide  
10 having the sequence as set forth in SEQ ID NO:4;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

12. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a lycopene cyclase enzyme of at least 389 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6;

20 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

13. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene desaturase enzyme of at least 492 amino acids that has at least 82% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the  
25 sequence as set forth in SEQ ID NO:8;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

14. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a phytoene synthase enzyme of at least 308 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:10;

30 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

15. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a  $\beta$ -carotene hydroxylase enzyme of at least 185 amino acids that has at least 73% identity based on the Smith-Waterman

method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:12;

or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

5           16. A chimeric gene comprising the isolated nucleic acid molecule of any one of Claims 4 or 10-15 operably linked to suitable regulatory sequences.

          17. A vector comprising the isolated nucleic acid molecule of Claim 6.

10           18. A transformed host cell comprising the chimeric gene of Claim 16.

          19. A transformed host comprising the isolated nucleic acid molecule of claim 6.

15           20. The transformed host cell of Claim 18 or 19 wherein the host cell is selected from the group consisting of bacteria, yeast, filamentous fungi, algae, and green plants.

          21. The transformed host cell of Claim 18 or 19 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*,  
20   *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*, *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*,  
25   *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*, *Methanobacterium*, *Klebsiella*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, *Torulopsis*, *Rhodotorula*, and *Phaffia*.

30           22. A method for the production of carotenoid compounds comprising:

          (a) providing a transformed host cell comprising:

          (i) suitable levels of farnesyl pyrophosphate; and  
          (ii) a nucleic acid molecule encoding carotenoid  
35           biosynthetic pathway enzymes under the control of suitable regulatory sequences, wherein the genetic organization of the molecule is: *crtE-idi-crtY-crtI-crtB-crtZ*, wherein the *crtE*, *idi*, *crtY*, *crtI*, *crtB*, and

*crtZ* genes are clustered in the order stated and wherein the transcription of the *crtZ* occurs in opposite orientation to that of *crtE*, *idi*, *crtY*, *crtI*, and *crtB*; and

- 5 (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is produced.

23. A method for the production of carotenoid compounds  
10 comprising:  
    (a) providing a transformed host cell comprising:  
        (i) suitable levels of farnesyl pyrophosphate; and  
        (ii) a set of nucleic acid molecules encoding the  
            enzymes selected from the group consisting of SEQ  
15 ID NOs:2, 4, 6, 8, 10, and 12 under the control of  
            suitable regulatory sequences;  
    (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is  
20 produced.

24. A method for the production of carotenoid compounds  
comprising:  
    (a) providing a transformed host cell comprising:  
        (i) suitable levels of farnesyl pyrophosphate; and  
25       (ii) a the isolated nucleic acid molecule of claim 6 under  
            the control of suitable regulatory sequences;  
    (b) contacting the host cell of step (a) under suitable growth conditions with an effective amount of a fermentable carbon substrate whereby a carotenoid compound is  
30 produced.

25. A method according to any one of Claims 22 – 24 wherein the transformed host cell is selected from the group consisting of C1 metabolizing hosts, bacteria, yeast, filamentous fungi, algae, and green plants.

- 35 26. A method according to Claim 25 wherein the C1 metabolizing host is a methanotroph and the fermentable carbon substrate is selected from the group consisting of methane, methanol, formaldehyde, formic acid, methylated amines, methylated thiols, and carbon dioxide.

27. A method according to Claim 26 wherein the C1 metabolizing host:

- (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- 5 (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate-dependent phosphofructokinase enzyme.

28. A method according to Claim 27 wherein the C1 metabolizing host cell is a high growth methanotrophic bacterial strain, known as  
10 *Methylomonas* 16a and having the ATCC designation PTA 2402.

29. A method according to Claim 25 wherein the transformed host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Yarrowia*, *Rhodospiridium*, *Lipomyces*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Zymomonas*,  
15 *Agrobacterium*, *Flavobacterium*, *Rhodobacter*, *Rhodococcus*, *Streptomyces*, *Brevibacterium*, *Corynebacteria*, *Mycobacterium*, *Escherichia*, *Pantoea*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Methylococcus*, *Methylosinus*, *Methylomicrobium*, *Methylocystis*, *Alcaligenes*, *Synechocystis*, *Synechococcus*, *Anabaena*, *Thiobacillus*,  
20 *Methanobacterium*, *Klebsiella*, *Methylophilus*, *Methylobacillus*, *Methylobacterium*, *Hyphomicrobium*, *Xanthobacter*, *Paracoccus*, *Nocardia*, *Arthrobacter*, *Rhodopseudomonas*, *Torulopsis*, *Rhodotorula* and *Phaffia*.

30. A method according any one of Claims 22 – 24, wherein the  
25 carotenoid compound produced is selected from the group consisting of: antheraxanthin, adonirubin, adonixanthin, astaxanthin, canthaxanthin, capsorubrin,  $\beta$ -cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, epsilon-carotene, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone,  $\gamma$ -carotene, 4-keto- $\gamma$ -carotene,  $\zeta$ -carotene,  $\alpha$ -cryptoxanthin, deoxyflexixanthin,  
30 diatoxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, isorenieratene, lactucaxanthin, lutein, lycopene, myxobactone, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, 4-keto-rubixanthin, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, 4-keto-torulene, 3-hydroxy-4-  
35 keto-torulene, uriolide, uriolide acetate, violaxanthin, zeaxanthin- $\beta$ -diglucoside, and zeaxanthin.

31. A method of regulating carotenoid biosynthesis in an organism comprising over-expressing at least one carotenoid biosynthetic pathway

gene selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11 and 18 in an organism such that the carotenoid biosynthesis is altered in the organism.

5        32. A method according to Claim 31 wherein said carotenoid gene is over-expressed on a multicopy plasmid.

      33. A method according to Claim 31 wherein said carotenoid gene is operably linked to an inducible or regulated promoter.

      34. A method according to Claim 31 wherein said carotenoid gene is expressed in antisense orientation.

10       35. A method according to Claim 31 wherein said carotenoid gene is disrupted by insertion of foreign DNA into the coding region.

      36. A strain DC404 comprising the 16s rDNA sequence as set forth in SEQ ID NO:16.